



Surveys for Pathogens of Monoecious Hydrilla in 2013

by Judy F. Shearer

PURPOSE: This technical note describes 2013 survey results for pathogenic agents on monoecious hydrilla.

INTRODUCTION: There are two biotypes of *Hydrilla verticillata* (L.f.) Royle (hydrilla) in the United States. The pistillate dioecious hydrilla biotype was introduced from Sri Lanka into Florida in the 1950's (Schmitz et al. 1990). It has spread throughout the Southeast United States, as far west as Texas and into parts of California (Madeira et al. 2004). Monoecious hydrilla was first discovered in Delaware in 1976 and later in the Potomac River (Haller 1982, Steward et al. 1984). It has now expanded its distribution through the Atlantic States and northward to Maine (Madeira et al. 2004). Separate populations have been reported in Iowa, Ohio, Indiana, Wisconsin, Kansas, Missouri, California, and Washington State (Nonindigenous Aquatic Species (NAS) 2011). The Washington State population no longer exists due to an aggressive eradication program.¹ It is believed that populations in Iowa and Wisconsin have also been eradicated.² Recent invasions have appeared in Lake Cayuga and at Tonawanda Creek/Erie Canal, both in upstate New York (Cornell Cooperative Extension (CCE) 2011, Lansing Star 2012). Shortly after its discovery in 1982, Steward et al. (1984) predicted that monoecious hydrilla had the potential to invade all of the lower 48 states and southern and central Canada. Balciunas and Chen (1993), after surveying for biocontrol agents in Asia and examining herbaria in the region, also indicated that hydrilla could become widespread in North America, including Canada and parts of Alaska. Although not known to exist in Minnesota, Maki and Galatowitsch (2008) ran a CLIMEX model (a software program that predicts the effect of climate change on species distribution) that indicated the state was at risk for invasion of monoecious hydrilla.

The growth forms of dioecious and monoecious hydrilla biotypes are different. Compared to the monoecious biotype, dioecious plants tend to have more vigorous growth extending vertically to the water surface then spreading laterally and forming a mat (Van 1989). Madeira et al. (1997) hypothesized that this growth form was an adaptation to deep water generated from monsoons on the Indian subcontinent. Tubers (i.e. vegetative propagules) can be found up to 30 cm deep in the sediment (Langeland 1996) but in North Carolina, Harlan et al. (1985) found the majority of monoecious tubers at depths of 0 to 8 cm in the sediment. Tubers of the dioecious biotype are larger than those of the monoecious biotype (Spencer et al. 1987) and are formed under short-day conditions (Van 1989). In contrast, tubers of the monoecious biotype are produced under long-day photoperiods and are smaller

¹Personal Communication. 2012. J. Parsons, Aquatic Plant Specialist, State of Washington Department of Ecology, Olympia, WA.

²Personal Communication. 2013. M. Netherland, Research Biologist, U. S. Army Engineer Research and Development Center, Gainesville, FL.

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(Spenser et al. 1987). When they sprout, stems tend to grow laterally and generate new root crowns along the sediment surface resulting in high shoot densities (Van 1989). When the monoecious hydrilla mat declines in the fall, it breaks loose and fragments containing numerous axillary propagules (i.e. turions) drift in water currents dispersing the plant (Steward and Van 1987). Madeira et al. (1997) hypothesized that this growth habit suggested a temperate origin of the plant that was consistent with its probable Korean origin.

While dioecious hydrilla has been surveyed for pathogenic agents periodically over the past 25 years (Joye and Cofrancesco 1991, Shabana and Charudattan 1996, Shabana et al. 2003, Shearer 2012), monoecious hydrilla has received little attention. In part, this was due to its limited distribution in a few eastern states, but the expansion of monoecious hydrilla in recent years to widely different geographic regions of the United States has given it new status as an invasive species of note.

Monoecious hydrilla management is primarily through chemical control using endothall (Poovey and Getsinger 2010), fluridone, and a combination of copper and diquat.¹ Grass carp (*Ctenopharyngodon idella*) are a potential biological control agent as a non-specific feeder of aquatic plants and in all likelihood would feed on monoecious hydrilla. Grass carp have been released in Lake Gaston along the North Carolina/Virginia border where they have tried to maintain target population densities of 3.2 to 20.5 fish per hydrilla-ha; however, there has been little evidence to show that grass carp have contributed to hydrilla control in the lake (Dick et al., in review). While the ephydrid fly *Hydrellia pakistanae*, a biocontrol insect, has successfully established populations on the dioecious hydrilla biotype, there are no published records of establishment on the monoecious hydrilla biotype even after concerted release efforts. Both greenhouse and outdoor-pond studies have documented that *Hydrellia* flies have reduced survival rates and longer developmental times on monoecious than dioecious hydrilla (Grodowitz et al. 2010). In north Texas, *Hydrellia* flies survive and overwinter as larval stages in stems of dioecious hydrilla (Harms and Grodowitz 2011). This overwintering survival mechanism may preclude fly establishment on monoecious hydrilla populations because the plant survives as subterranean tubers and turions (Steward and Van 1987) and not as aboveground biomass.

Several pathogens have been researched as potential biological control agents for management of dioecious hydrilla, including *Mycoleptodiscus terrestris* (Joye and Cofrancesco 1991; Joye 1990; Joye and Paul 1991; Nelson et al. 1998; Netherland and Shearer 1996; Shearer 1998; Shearer 2009a, 2009b; Shearer and Nelson 2002; Shearer and Jackson 2006), *Fusarium culmorum* (Charudattan et al. 1984), and *Plectosporium tabacinum* (Smither-Kopperl et al. 1999). To date, no pathogens have been researched as potential biological control agents for management of monoecious hydrilla. The purpose of the study presented herein was to survey some known populations of monoecious hydrilla and isolate potential fungal pathogens.

MATERIALS AND METHODS: During the summer/fall of 2013, monoecious hydrilla was collected in the field from Strom Thurmond Reservoir in South Carolina/Georgia, Lake Guntersville in Alabama, and Lake Cayuga and Tonawanda Creek/Erie Canal in upstate New York. Samples were also received from the Center for Aquatic and Invasive Plants (CAIP) in Gainesville, Florida where monoecious hydrilla plants collected in Missouri, Kansas, South Carolina/Georgia (Strom Thurmond Reservoir), and North Carolina/Virginia (Lake Gaston) had been cultured. All samples were shipped overnight to

¹Netherland, M. 2013. Research Biologist. U.S. Army Engineer Research and Development Center, Gainesville, FL.

the biomanagement laboratory located at the U.S. Army Engineer Research and Development Center (USAERDC) in Vicksburg, Mississippi. Upon arrival, samples were washed in running water to remove any soil or debris attached to stems and leaves. Samples were wrapped in moist paper toweling, placed in plastic bags, and kept at 4 °C until they could be processed.

Samples were processed by dilution plating. A 10-g subsample of stem and leaf tissue from each collection was surface sterilized in a 3.5% sodium hypochlorite solution for 1 min, placed in a sieve, and rinsed in deionized water for 1 min. Excess moisture was drained off the subsample and it was added to a sterile blender containing 100 ml of sterile water. The subsample was macerated in the blender for 30 sec, providing a dilution factor of 1/10. The resulting slurry was further diluted to concentrations of 1/50 and 1/100. All dilutions were plated onto Martin's agar (1 L H₂O; 20 g agar, 0.5 g KH₂PO₄; 0.5 g MgSO₄·7 H₂O; 0.5 g peptone; 10 g dextrose, 0.5 g yeast extract; 0.05 g rose Bengal; 0.03 g streptomycin sulfate) plates (three plates per dilution concentration). Plates were incubated in the dark at 25 °C for 1 week. Small pieces (~1- by 1-mm) were cut from the leading edge of filamentous fungal colonies on the plates and transferred to Potato Dextrose Agar (PDA; Difco Inc., Detroit, Michigan) slants (test tubes placed at an angle during cooling to give a large slanted surface for inoculation). After 7-10 days, slants from each sample location were sorted together and enumerated into morphospecies based on gross colony morphology and color. Each morphological "species" was plated onto Potato Carrot Agar (PCA; Dhingra and Sinclair 1995) and PDA and incubated at 25 °C under a grow light (plant aquarium wide spectrum) (General Electric, Fairfield, Connecticut) for 1 to 3 weeks to induce sporulation. Both agars are important for isolate identification because characteristic colors and growth patterns develop on PDA, and colonies readily produce asexual and/or sexual spores on PCA. Cultures that sporulated were identified to genus and species when possible. Those that did not sporulate were placed in categories of moniliaceous (hyaline hyphae) or dematiaceous (dark hyphae) Ascomycetes. Each isolate was also transferred to a one-half strength corn meal agar (Difco Inc., Detroit, Michigan) slant, allowed to grow for 2 weeks, and then placed in cold storage at 4 °C.

Because monoecious hydrilla shipments arrived at different intervals during the summer and fall of 2013, and were sorted separately, it could not be discerned if the cultures designated as *Penicillium* sp., *Phoma* sp., moniliaceous or dematiaceous Ascomycetes were the same or different across shipments. Therefore, the isolates were replated onto PDA and compared for morphological similarity and color so that equates could be made.

RESULTS AND DISCUSSION: The number of morphospecies isolated from Lake Cayuga, Tonawanda Creek/Erie Canal, Lake Guntersville, Strom Thurmond Reservoir, and from culture tank plants that originated at Strom Thurmond Reservoir, Kansas, Missouri, and Lake Gaston was 25, 20, 29, 21, 21, 15, 26, and 18, respectively. Following identification of the morphospecies, the total number of species from all the samples was 110 (Table 1). Of the total number of species isolated from the samples, 75 were singletons (occurred in one sample), 22 were doubletons (occurred in two samples), 7 were tripletons (occurred in three samples), and 6 species were isolated from four or more samples. These were *Cladosporium sphaerospermum*, *Trichoderma harzianum*, *Pestalotiopsis guepinii*, *Microsphaeropsis olivacea*, *Phoma nebulosa*, and *Phoma leveillei*. All six are ubiquitous species that are isolated from a variety of substrates including soil and organic matter.

Table 1. Fungi isolated from monoecious hydrilla collections in 2013.

Species	Cayuga	Erie	Lake Guntersville	Strom Thurmond Field	Strom Thurmond Culture	Kansas	Missouri	Lake Gaston
<i>Pseudotorula heterospora</i>	x						x	
Moniliaceous Ascomycete 1	x							
<i>Pythium</i> sp.	x					x		x
<i>Alternaria alternata</i>	x		x				x	
<i>Gliocladium</i> sp.	x							
<i>Curvularia lunata</i>	x							
<i>Aposphaeria pulviscula</i>	x							
<i>Microsphaeropsis olivacea</i>	x	x		x		x	x	
<i>Plectosphaerella cucumerina</i>	x	x						
<i>Ascochyta pisi</i>	x							
<i>Cylindrocarpon tunue</i>	x							x
<i>Phoma leveillei</i>	x	x	x	x				
<i>Phoma</i> sp. 1	x							
<i>Fusarium sambucinum</i>	x							
<i>Trichoderma hamatum</i>	x	x						
Dematiaceous Ascomycete 1	x							
<i>Acremonium potronii</i>	x			x				
<i>Fusarium heterosporium</i>	x							
<i>Verticicladium trifidum</i>	x							
<i>Trichoderma piluliferum</i>	x							
<i>Cladosporium sphaerospermum</i>	x		x		x	x	x	x
<i>Trichoderma harzianum</i>	x	x	x	x		x	x	x
Moniliaceous Ascomycete 2	x							
<i>Cladosporium cladosporioides</i>	x		x	x				
Moniliaceous Ascomycete 3	x							
<i>Sporormiella minimoides</i>		x						
Moniliaceous Ascomycete 4		x						
Dematiaceous Ascomycete 2		x					x	
<i>Trichoderma aureoviride</i>		x	x					
<i>Penicillium</i> sp. 1		x						

Table 1. Fungi isolated from monoecious hydrilla collections in 2013.

Species	Cayuga	Erie	Lake Guntersville	Strom Thurmond Field	Strom Thurmond Culture	Kansas	Missouri	Lake Gaston
<i>Selenophoma uncea</i>		x						
<i>Tritirachium</i> sp.		x		x				
<i>Acremonium charticola</i>		x	x					
<i>Acremonium pteridii</i>		x						
Dematiaceous Ascomycete 3		x						
<i>Phomopsis</i> sp.		x						
<i>Graphium penicilloides</i>		x						
<i>Colletotrichum gloeosporioides</i>		x	x					
<i>Penicillium</i> sp. 2		x	x					
<i>Arthrinium sphaerospermum</i>		x		x				
<i>Phoma</i> sp. 2			x					
<i>Phoma nebulosa</i>			x	x	x		x	
Dematiaceous Ascomycete 4			x					
<i>Nodulisporium ochraceum</i>			x					
Dematiaceous Ascomycete 5			x					
<i>Epicoccum purpurascens</i>			x					
<i>Penicillium</i> sp. 3			x					
Moniliaceous Ascomycete 5			x					
<i>Cladosporiella cercosporicola</i>			x					
<i>Rhinocladiella cellaris</i>			x					
<i>Periconia bysoides</i>			x					
<i>Penicillium</i> sp. 4			x			x	x	
<i>Sclerotium rolfsii</i>			x					
<i>Emericellopsis minima</i>			x	x				
<i>Umbellopsis</i> sp.			x					
<i>Dactylella</i> sp.			x					
<i>Penicillium multicolar</i>			x					
<i>Sphaeropsis sapinea</i>			x					
<i>Pestalotiopsis guepinii</i>			x	x	x		x	x
Dematiaceous Ascomycete 6			x					
<i>Penicillium</i> sp. 5				x				

Table 1. Fungi isolated from monoecious hydrilla collections in 2013.

Species	Cayuga	Erie	Lake Guntersville	Strom Thurmond Field	Strom Thurmond Culture	Kansas	Missouri	Lake Gaston
<i>Penicillium purpurogenum</i>				x				x
<i>Graphium caliciooides</i>				x				
<i>Pyrenophaeta</i> sp.				x				
Moniliaceous Ascomycete 6				x				
<i>Fusarium oxysporum</i>				x				
<i>Penicillium</i> sp. 6				x				
Dematiaceous Ascomycete 7				x				
<i>Penicillium</i> sp. 7				x				
Oomycota sp.				x				
Moniliaceous Ascomycete 7				x				
<i>Myrothecium roridum</i>					x	x	x	
Moniliaceous Ascomycete 8						x		
<i>Curvularia geniculata</i>						x		
<i>Aspergillum terreus</i>					x	x		
<i>Mycoleptodiscus</i> sp.						x		
<i>Coelophoma cylindrospora</i>						x		x
Dematiaceous Ascomycete 8						x		
<i>Graphium putredinis</i>						x		
<i>Aspergillum niger</i>							x	
<i>Pithomyces maydicus</i>					x		x	
<i>Curvularia senegalensis</i>						x	x	x
<i>Cylindrocarpon</i> sp.							x	
<i>Penicillium</i> sp. 7					x		x	x
<i>Phoma</i> sp. 2							x	
<i>Humicola fuscoatra</i>							x	
<i>Phoma</i> sp. 3							x	
<i>Fusarium lateritium</i>							x	
<i>Rhizopus nigricans</i>							x	
<i>Botrytis</i> sp.							x	
<i>Metarrhizium anisopliae</i>							x	
Moniliaceous Ascomycete 9							x	
Moniliaceous Ascomycete 10							x	

Table 1. Fungi isolated from monoecious hydrilla collections in 2013.

Species	Cayuga	Erie	Lake Guntersville	Strom Thurmond Field	Strom Thurmond Culture	Kansas	Missouri	Lake Gaston
<i>Penicillium variable</i>						x	x	
Dematiaceous Ascomycete 9							x	
Moniliaceous Ascomycete 11					x			
Dematiaceous Ascomycete 10					x			
<i>Phoma</i> sp. 4					x			
<i>Nodulisporium gregarium</i>					x			x
<i>Spegazzinia deightonii</i>					x			
<i>Pithomyces chartarum</i>					x			x
<i>Periconia atra</i>					x			
<i>Pithomyces graminicola</i>					x			
<i>Verticillium</i> sp.					x			
<i>Paecilomyces carneus</i>								x
Moniliaceous Ascomycete 12								x
<i>Penicillium</i> sp. 8								x
Moniliaceous Ascomycete 13								x
<i>Geotrichum</i> sp.								x
<i>Acremonium humicola</i>								x

It is not unusual to have a high number of singletons in each sample. In most cases, about half the number of species isolated from each sample are singletons. In the study completed in 2012 (Shearer 2014), singletons and doubletons accounted for 81% of the total species. In the present study, it was approximately 89%. Two factors that could potentially account for the high number of singletons are site differences (water quality, plant nutrition, plant maturity, geographic location, etc.) and the number of samples from each site. Typically it is difficult to handle more than four or five samples from a site simply because each sample can yield a large number of morphospecies. Too many isolates can make processing unmanageable.

Comparing 2012 with 2013 collections, there were only eight species in common: *Alternaria alternata*, *Microsphaeopsis olivacea*, *Plectosphaerella cucumerina*, *Cladosporium sphaerospermum*, *C. cladosporoides*, *Trichoderma harzianum*, *Phoma nebulosa*, and *Emericellopsis minima*. All of these species have been isolated with regularity from hydrilla collections from different geographic regions of the United States. In that regard, they would be considered cosmopolitan species. The *Penicilliums* were not identified to species because they are ubiquitous saprobes and therefore are not good biological control agents. Also, it was not a good use of time to plate the *Penicilliums* on special media for identification purposes. For these reasons, there may actually have been more species in common

than the eight identified above. The moniliaceous and dematiaceous Ascomycetes were not identified and compared across years for morphological similarities.

Collections were made at Strom Thurmond Reservoir in 2012 and 2013, but not at the same locations. *Cladosporium sphaerospermum* was the only species found to be in common between the two years. Strom Thurmond field collections from 2013 were also compared with cultured material sent from the CAIP. There were three species in common: *P. nebulosa*, *C. sphaerospermum*, and *Pestalotiopsis guepinii*. Although *P. guepinii* was not isolated from Strom Thurmond 2012 collections, it too is a cosmopolitan species that occurs with regularity in hydrilla plant samples.

The majority of species isolated during the study could be described as cosmopolitan saprobes, or secondary weak pathogens, and as such would not make good candidates for biological control of monoecious hydrilla. Potential exceptions could be *Myrothecium roridum*, *Plectosphaerella cucumerina*, *Mycoleptodiscus* sp., *Colletotrichum gloeosporioides*, *Fusarium* sp. and *Verticillium* sp.

Myrothecium roridum has been suggested as a possible mycoherbicidal agent for control of waterhyacinth (Okunowo et al. 2010b). The published paper focused on optimum growth parameters of the fungus but efficacy testing on waterhyacinth was not included. Because species of *Myrothecium* can produce cellulolytic enzymes, *M. roridum* might have potential as a pathogenic agent (Moreira et al. 2005; Okunowo et al. 2010a) both for waterhyacinth and monoecious hydrilla. In screening of potential pathogens of monoecious hydrilla collected in 2012, *M. roridum* was found to be extremely efficacious, with a disease rating of 3.2 out of a maximum of 4.¹

In the late 1990s, Smith-Kopperl et al. (1999) isolated *Plectosporium tabacinum* (anamorph *Plectosphaerella cucumerina*) from asymptomatic dioecious hydrilla. In an aquarium study, the fungus could spread to other plants from a single infected shoot. However, it was considered weakly pathogenic and the authors recommended it be used with herbicides in an integrated approach for hydrilla management. This approach might also be applied when using *P. cucumerina* as a biocontrol fungus for monoecious hydrilla management.

Species in the genus *Mycoleptodiscus* have been reported as pathogens on a number of plants (Sutton and Hodges 1976, Smith et al. 1998). Specifically, *M. terrestris* has been found to be efficacious on dioecious hydrilla used alone (Joye 1990; Shearer and Jackson 2006; Shearer 1998, 2009a, 2012) and in combination with herbicides (Netherland and Shearer 1996, Shearer and Nelson 2002, Nelson and Shearer 2009). The isolation of another *Mycoleptodiscus* species from monoecious hydrilla during the 2013 surveys indicates that it can invade host tissues. Future testing will determine if the isolate is efficacious on the monoecious hydrilla biotype.

Colletotrichum species cause anthracnose on a variety of annual crop and ornamental plants (Agrios 2005, d'A. Charchar 2002, Jiang et al. 2012, Than et al. 2008). One of the most common species, *C. gloeosporioides*, has been associated with over 470 host genera (Hyde et al. 2009). Some strains are highly host specific and have been developed as bioherbicides in several regions of the world. For example, *C. gloeosporioides* f. sp. *aeschynomene* has been developed as the mycoherbicide

¹ Unpublished data. 20121 Dr. Judy Shearer, Research Plant Pathologist, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Collego™ (Templeton 1986) for management of *Aeschynomene virginica* (Northern joint vetch) in rice, and *C. gloeosporioides* f. sp. *malvae* has been developed as the mycoherbicide Biomal® for management of *Malva pusilla* (round-leaved mallow) in prairie agriculture (Boyetchko et al. 2007). If *C. gloeosporioides* isolated during the 2013 surveys is efficacious on monoecious hydrilla, it could be similarly formulated as a mycoherbicide.

Species in the genera *Fusarium* and *Verticillium* are best known as vascular wilt pathogens of vegetables, flowers, field crops, perennial ornamentals, and some fruit and forest trees (Agrios 2005). Wilting occurs when the fungus invades xylem tissues of the plant. As a result, herbaceous plants may die within just a few weeks while it may be a matter of months or years for perennial plants. Most wilts in the genus *Fusarium* are attributed to the species *F. oxysporum*. Within the species complex are strains or races of the fungus that are host specific. For example, *F. oxysporum* f. sp. *lycopersici* attacks only tomatoes, *F. oxysporum* f. sp. *conglutinans* attacks only cucurbits, and *F. oxysporum* f. sp. *cubense* attacks only bananas (Agrios 2005). The causal agents of most *Verticillium* wilts are *V. albo-atrum* and *V. dahlia*. Although both species have been reported to have a broad host range, attacking over 200 species of plants, some strains can be host specific (Agrios 2005) (e.g. *V. albo-atrum* var. *menthae* attacks *Mentha* (mint) species) (Farr et al. 1989). *Verticillium albo-atrum* and *V. dahlia* are difficult to distinguish, but in general *V. dahlia* does not grow at temperatures exceeding 30 °C. Further culturing is required to determine which species was isolated from monoecious hydrilla in this study.

FUTURE WORK: The above-mentioned potential pathogens should be screened for pathogenicity on monoecious hydrilla to determine if any of them are biological control agent candidates. The first step in the evaluation process is to do a flask study in which the pathogens are grown in broth culture and inoculated onto apical tips of hydrilla. All the unknown dematiaceous and moniliaceous Ascomycetes and any isolate identified as a weak pathogen should also be included in the test list. Those isolates identified as cosmopolitan saprobic species would not be tested. Depending on the results of the flask test, the next step would be a scale-up to an aquarium study.

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